Automated determination of the uniformity of dosage in quinine sulfate tablets using a fiber optics autosampler

Application Note

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Introduction

Conventional automated analysis of pharmaceutical products by UV-Vis spectroscopy has involved the use of routine samplers or sippers. The introduction of Agilent's fiber-optics autosampler has provided a means of dramatically decreasing the analysis time and minimizing sample usage.

By the use of a concentration application module with Cary OS2 software and a simple ADL program to locate the peak maximum and its absorbance value, the concentration of the analyte can be determined from known standards or a known extinction coefficient providing a simple, fast routine analytical procedure.

This has been applied to the measurement of uniformity of dosage for quinine sulfate in tablets using the USP procedure.

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Experimental

Apparatus

- Cary 3C Double Beam UV/Vis Spectrophotometer.
- Agilent SPS-5 Autosampler
- Cary Fiber-Optics Probe and Fiber Optics Coupler.
- Cary OS2 Concentration Application

Reagents

- Quinine sulfate reference standard.
- Dilute hydrochloric acid (1:100 Analytical Reagent grade hydrochloric acid in distilled deionized water)

ADL program

The analysis described requires that the peak maximum be used, where the maximum absorbance occurs within a defined range.

An ADL program is run for each sample. This program is activated from the "USER COLLECT" function in the instrument setup dialog.

The program carries out a wavelength scan from 400 to 320 nm, then finds the peak maximum, checking that it occurs within the required limits, then returns the absorbance for the peak maximum to the Concentration application for inclusion in the calibration or sample data.

Appendix 2 lists the ADL program for Quinine sulfate.

Procedure

Standard Preparation

Bulk standards

Accurately weigh 0.2000 gram of quinine sulfate and dilute to 200.0ml with dilute hydrochloric acid (1 in 100).

Working standards

Prepare working calibration standards by accurately diluting the volumes of bulk standard to 100.00 mL with dilute hydrochloric acid (1 in 100) using the following table:

Table 1. Preparing working calibration standards

Tablet conc mg/tablet	100	200	300	400
mL of bulk standard/100 mL	12.00	24.00	36.00	48.00

Sample preparation

1. Select 10 tablets and transfer each to an individual 250 mL volumetric flask, add about 175 mL of dilute hydrochloric acid (1 in 100) to each, and shake by mechanical means for 30 minutes. Make up to volume, and mix.
2. Filter a portion of the mixture (grade 42 filter paper), discard the first 20 mL and collect the remainder.
3. Further dilute 3.00 mL of this to 100.00 mL in a volumetric flask making to the mark with dilute hydrochloric acid (1 in 100).
4. Transfer to the required number of test tubes in rack 1 of the SPS-5 sampler.
5. Fill the standards rack tubes with the above standards located in positions 1 through 4 and load a blank solution of dilute hydrochloric acid (1 in 100) in position 12.

Analysis procedure

1. Load the concentration software and recall the relevant method. (Refer Appendix 3)
2. It is necessary to collect a system baseline by running baseline from the BASE system and storing this baseline with the method. The ADL then uses this baseline during analysis. Use the ADL command "ADS_SHAKE(n,m,10,1)" to move the sample probe arm to vial "n", rack "m" where the analytical blank is located to record the baseline.
3. Load the sample names either by cutting and pasting from an existing sample list or enter in the advanced sample names dialog.
4. Highlight the sample names to be analyzed, and press READ.
5. The sampler arm will first do an align procedure, rinse, then proceed to the samples.

As the carry-over between samples is very low, a rinse between like samples is not necessary. The shake programmed into the fiber-probe will effectively remove all excess drops from the fiber optic probe tip before the probe moves to the next tube.

Figure 1 shows the autosampler setup dialogs, where the user can define the rack and tube positions for the samples and standards. **Note** that it is possible to use the same rack for both standards and samples, with correct selection of start tube position.

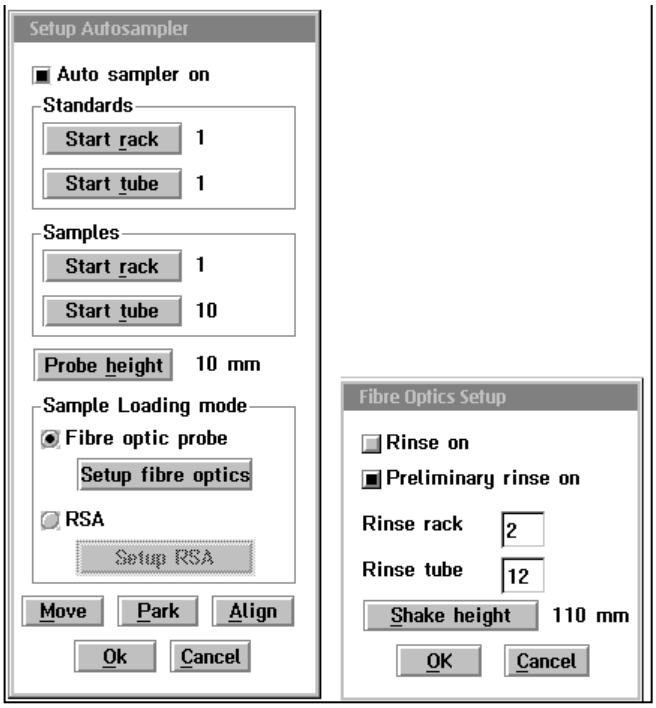

Figure 1. Fiber optic setup dialogs

Results

These results illustrate the typical absorbance and concentration values obtainable.

The results shown in Table 1 (see Appendix 1) were collected for 10 individual tablets from one bottle and illustrate the excellent precision attainable with the fiber optic autosampler. The standard deviation and %RSD were obtained from 3 replicates of each sample. The excellent precision indicates the ability of the fiber optic probe to work in the ambient environment shielded by the sampler covers. The linearity of the calibration is indicated by the correlation coefficient of 0.99998 can be seen by the excellent fit of the actual data points to the calibration line.

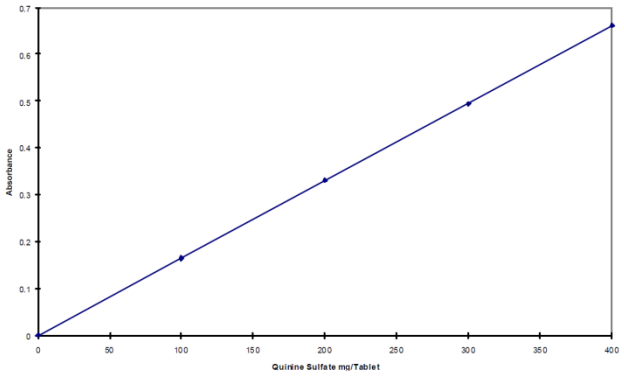

Figure 2. Quinine sulfate calibration graph

The data are reported into the Cary report generator as shown in Appendix 2, where all instrument operating conditions and the date/ and time of analysis are recorded.

The software graphical window shows the results where the user can incorporate action and warning limit lines to monitor the product quality.

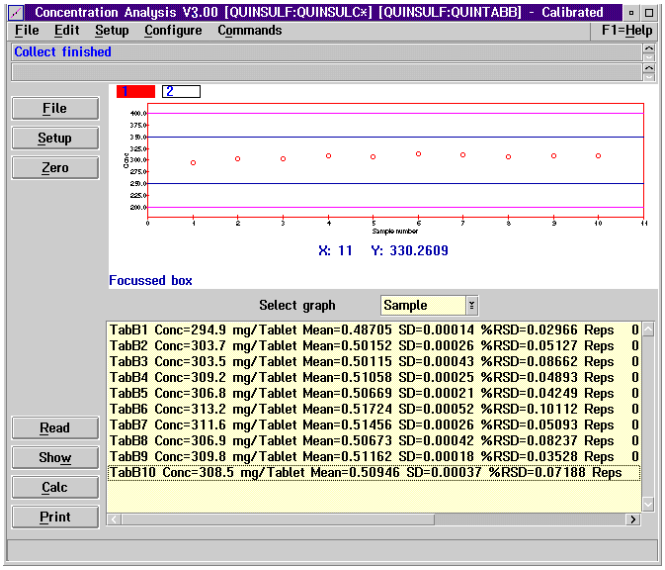
Concentration Analysis V3.00 [QUINSULF:QUINSULC*] [QUINSULF:QUINTABB] - Calibrated
File Edit Setup Configure Commands F1=Help
Collect finished
File
Setup
Zero
X: 11 Y: 330.2609
Focussed box
Select graph Sample
TabB1 Conc=294.9 mg/Tablet Mean=0.48705 SD=0.00014 %RSD=0.02966 Reps 0
TabB2 Conc=303.7 mg/Tablet Mean=0.50152 SD=0.00026 %RSD=0.05127 Reps 0
TabB3 Conc=303.5 mg/Tablet Mean=0.50115 SD=0.00043 %RSD=0.08662 Reps 0
TabB4 Conc=309.2 mg/Tablet Mean=0.51058 SD=0.00025 %RSD=0.04893 Reps 0
TabB5 Conc=306.8 mg/Tablet Mean=0.50669 SD=0.00021 %RSD=0.04249 Reps 0
TabB6 Conc=313.2 mg/Tablet Mean=0.51724 SD=0.00052 %RSD=0.10112 Reps 0
TabB7 Conc=311.6 mg/Tablet Mean=0.51456 SD=0.00026 %RSD=0.05093 Reps 0
TabB8 Conc=306.9 mg/Tablet Mean=0.50673 SD=0.00042 %RSD=0.08237 Reps 0
TabB9 Conc=309.8 mg/Tablet Mean=0.51162 SD=0.00018 %RSD=0.03528 Reps 0
TabB10 Conc=308.5 mg/Tablet Mean=0.50946 SD=0.00037 %RSD=0.07188 Reps
Read
Show
Calc
Print

Figure 3. Concentration application with sample and control lines

The analytical data can also be transferred by a number of paths to spreadsheets and other data manipulation programs.

DDE can be activated to transfer the results directly into a spreadsheet or compatible program, the data transferred contain the sample or standard number, the concentration and the box number.

Alternatively the sample list is a comma-delimited list that scan be imported directly into a spreadsheet and provides all the data seen in the results window, including past history that may be required for GLP records.

References

1. U.S. Pharmacopeia National Formulary USP XXIII NF XXIII 22 Edition Pages 1205.

Disclaimer

This publication is intended to illustrate a typical application and is not intended to be a definitive procedure or protocol. The results do not reflect any actual control or test samples. Users are advised to ensure they adapt the method to comply with their own protocols.

Appendix 1

	Average Absorbance	Standard Deviation	%RSD	mg/Tablet	Tablet Weight g	mg/g
Tablet 1	0.48705	0.000143	0.03%	294.94	0.7145	412.80
Tablet 2	0.50152	0.00026	0.05%	303.70	0.7382	411.40
Tablet 3	0.50115	0.000433	0.09%	303.48	0.7124	425.99
Tablet 4	0.51058	0.000251	0.05%	309.18	0.7384	418.72
Tablet5	0.50669	0.000213	0.04%	306.83	0.6834	448.97
Tablet 6	0.51723	0.000524	0.10%	313.21	0.6905	453.60
Tablet 7	0.51455	0.000262	0.05%	311.59	0.7502	415.34
Tablet 8	0.50673	0.000417	0.08%	306.85	0.7308	419.89
Tablet 9	0.51162	0.000181	0.04%	309.81	0.6779	457.02
Tablet 10	0.50946	0.000367	0.07%	308.50	0.7227	426.88
Average	0.50666	0.00031	0.060%	306.81	0.72	429.06
StDev				5.19	0.02	17.48
%RSD				1.69%	3.49%	4.07%
Calibration equation	Abs = 0.00166 *Conc + 0.06458					
Correlation coefficient	0.99998					

Appendix 2

Example of printed report

Quinine Analysis 08-29-96 11:47 PM Page 1
Concentration Analysis Report

Instrument Settings

Operator	John Sanders
Method	QUINSULF:QUINSULF
Instrument	Cary 1/3
Base system version no.	3.00
Instrument version no.	0.00
Application	Concentration Analysis 3.00
Wavelength (nm)	400.00
Ordinate Mode	A
SBW (nm)	2.0
SAT (sec)	0.5333
Standard replicates	3
Sample replicates	3
Fit type	Linear
Min R2	0.95000
Warning limits	250.0, 350.0
Action limits	200.0, 400.0
Units	mg/Tablet

Comments :
Quinine Sulfate test runs for
Fibre Optics application

User collect	On
User_result =	executec("adl:quinine.adl")
Autosampler :	ON

Calibration

Standard	Conc	Flags	Mean
Std 1	100.0	r	0.23091
Std 2	200.0	r	0.39662
Std 3	300.0	r	0.56116
Std 4	400.0	r	0.72928

Standard	Time
Std 1	Thu Aug 29 23:49:18 1996
Std 2	Thu Aug 29 23:50:43 1996
Std 3	Thu Aug 29 23:52:06 1996
Std 4	Thu Aug 29 23:53:31 1996

Calibration eqn: Abs = 0.00166 * Conc + 0.06458
Quinine Analysis 08-29-96 11:47 PM Page 2
Correlation coefficient: 0.99998
Calibration time: Thu Aug 29 23:53:59 1996

Analysis

Sample List : QUINSULF:QUINSTAB

Sample	Conc	Flags	Mean
Tablet1	294.9		0.48705
Tablet2	303.7		0.50152
Tablet3	303.5		0.50115
Tablet4	309.2		0.51058
Tablet5	306.8		0.50669
Tablet6	313.2		0.51723
Tablet7	311.6		0.51455
Tablet8	306.9		0.50673
Tablet9	309.8		0.51162
Tablet10	308.5		0.50946

Sample	Time
Tablet1	Thu Aug 29 23:55:44 1996
Tablet2	Thu Aug 29 23:57:10 1996
Tablet3	Thu Aug 29 23:58:36 1996
Tablet4	Fri Aug 30 00:00:03 1996
Tablet5	Fri Aug 30 00:01:29 1996
Tablet6	Fri Aug 30 00:02:55 1996
Tablet7	Fri Aug 30 00:04:21 1996
Tablet8	Fri Aug 30 00:05:50 1996
Tablet9	Fri Aug 30 00:07:15 1996
Tablet10	Fri Aug 30 00:08:40 1996

Results Flags Legend

A+ = Above max. action limit A- = Below min. action limit
W+ = Above max. warning limit W- = Below min. warning limit
r = Repeat reading c = Recalculated result
u = Uncalibrated O = Overrange
* = Sample not measured with current parameters.

ADL Program

This listing details the ADL program written specifically for quinine sulfate analysis used by Cary OS/2 Concentration software to create the data required for the concentration application for the analysis.

Note: With minor modifications to the wavelength ranges, the ADL program can be used for a variety of pharmaceutical analysis.

Appendix 3

```
DEFINE QUININE_SULFATE
{Define variables}
        var curr_box
        var peak_found
        VT__USER_RESULT = 0
        curr_box = STDPLOT :active_box
        STDPLOT clear_box: curr_box
{Set-up instrument parameters}
        set(P_ABS_MODE, ABS_WAVLEN)                    {Set Wavelength Abscissa}
        set(P_ORD_MODE, ORD_ABS )                      {Set Absorbance ordinate}
        set(P_BASELINE_ONOFF,True)                     {Turn ON Baseline correction}
        set(XMAX,400)                                  {Set upper wavelength for scan}
        set(XMIN,320)                                  {Set lower wavelength for scan}
        set(YMAX,0.8)                                  {Set upper ordinate range}
        set(YMIN,0)                                    {set lower ordinate range}
        set(INTV,1.0)                                  {set data interval}
        COLLECT#                                       {Collect the continuum}
        a=SG_SMOOTH(result#,7)                         {smooth the continuum}
        DB[P_PEAK_DETECT]=0.005                        {Setting the peak threshold}
        DB[P_PEAK_MODE]=SRCH_PKS                       {Sets peak detect mode peaks and searches for peaks }
        PEAK(a,0)
        repeat
        peak_found = peak(a,1)
        if peak_found <> 0 then
        begin
        if (peak_x>320) and (peak_x <360) then  {set allowable wavelength range for peak max}
        VT__USER_RESULT=peak_y
        end
until peak_found = 0
return( VT__USER_RESULT )
set(P_BASELINE_ONOFF,False)
ENDDEF
```

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